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Tacoma, WA 98402

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14. ABSTRACT

Our overall hypothesis is that oral or intravenous resuscitation results in distinct improvements in burn-induced SIRS and AKI. Specifically, while oral resuscitation (i.e., drinking) helps in reducing SIRS, MOD and AKI post-burn injury, we predict it will not be as effective as the gold standard i.v. fluid resuscitation which may relate to fluid volume requirements that cannot be met orally. Moreover, we hypothesize that i.v. blood products (e.g., fresh frozen plasma) will improve organ perfusion and outcomes when compared to crystalloids, and thus reduce total fluid requirements. Resuscitation strategies will vary in ameliorating burn induced renal perfusion and dysfunction because of a differential effect on circulating cytokines and granulocytes. Subsequently, markers and byproducts of oxidative stress will increase as renal perfusion decreases. Information from the studies described in this proposal will elucidate what effect low volume post-burn resuscitation strategies have on the mechanisms of oxidative stress and systemic and local inflammation. This will not only provide information on the ensuing SIRS, MOD, and AKI, but also allow for future testing of therapies to modulate these mechanisms. The ultimate goal is to improve outcomes after extensive burn in austere environments where large volumes of fluid are not available and the casualty is delayed in transport to a treatment facility.

15. SUBJECT TERMS

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INTRODUCTION:
Our overall hypothesis is that oral or intravenous resuscitation results in distinct improvements in burn-induced SIRS and AKI. Specifically, while oral resuscitation (i.e., drinking) helps in reducing SIRS, MOD and AKI post-burn injury, we predict it will not be as effective as the gold standard i.v. fluid resuscitation which may relate to fluid volume requirements that cannot be met orally. Moreover, we hypothesize that i.v. blood products (e.g., fresh frozen plasma) will improve organ perfusion and outcomes when compared to crystalloids, and thus reduce total fluid requirements. Resuscitation strategies will vary in ameliorating burn induced renal perfusion and dysfunction because of a differential effect on circulating cytokines and granulocytes. Subsequently, markers and byproducts of oxidative stress will increase as renal perfusion decreases. Information from the studies described in this proposal will elucidate what effect low volume post-burn resuscitation strategies have on the mechanisms of oxidative stress and systemic and local inflammation. This will not only provide information on the ensuing SIRS, MOD, and AKI, but also allow for future testing of therapies to modulate these mechanisms. The ultimate goal is to improve outcomes after extensive burn in austere environments where large volumes of fluid are not available and the casualty is delayed in transport to a treatment facility.

KEYWORDS:
Burn, prolonged field care, enteral, rehydration salts, intravenous, resuscitation, swine models, crystalloid, colloid, third spacing

ACCOMPLISHMENTS:
The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

Specific Aim 1: Determine the effectiveness of gastroenteral resuscitation in mitigating SIRS, MOD and AKI. (0-10 months) – completed month 11

Objective 1a: Identify the effect of gastroenteral resuscitation on renal perfusion and AKI. (0-9 months)- completed month 11

Milestones: iACUC approval- (Months 0-1)- completed month 1

ACURO approval- (Months 1-3)- completed month 1

Objective 1b: Identify the effect of gastroenteral resuscitation on systemic and local inflammation. (6-10 months)- 86% complete- All 6 blood draw timepoints have been analyzed for 13 different cytokines, however local cytokines in the kidney have not been examined yet.

Milestones: Manuscript submission (11-13 months)- 90% complete. A draft is currently being rea and approved by all authors.

Specific Aim 2: Determine the effectiveness of limited volume i.v. resuscitation for mitigating SIRS, MOD and AKI. (10-21 months) – 10% complete- Animal experiments have been initiated.

Objective 2a: Compare the effects of instillation of lactated Ringer's (LR) as calculated by the modified Brooke Formula, versus a limited resuscitation volume paradigm. (10-21

months)- 40% complete. Animal experiments have been initiated, with approximately half of these groups completed.
Objective 2b: Compare the effects of LR with 2 different colloids: 5% albumin and fresh frozen plasma (FFP) on SIRS, MOD, and AKI. (13-21 months)- Not yet begun.

What was accomplished under these goals?

1) In the past year, we have completed a large number of animal experiments such that they are slightly ahead of schedule. This is advantageous, especially considering the longer than anticipated processing of tissues after experiments have been run. After 40%TBSA contact burns, animals have been recovered to metabolic cages in order to separate urine and feces, and fluid intake groups have been expanded to: 1- water deprivation, 2- ad libitum access to water, 3- volume-matched oral rehydration solution (ORS at 70mL/kg/d), and 4- a volume-limited (15mL/kg/d) ORS.
2) Because of the animal experiments described above, we are slightly ahead of schedule in that we have begun initial experiments using i.v. resuscitation. Again, this is likely to be offset by the processing times of tissues after experiments are run.
3) We have completed our objective in establishing the resuscitative importance of drinking fluids for improving kidney function post-burn.
4) Specifically, we found that the clinically-relevant AKI at 6 hours post-injury (as defined by KDIGO criteria) is ameliorated with enough volumes (70mL/kg/d water or ORS), but not with lower volumes of drinking fluids.
5) CT-angiographies have produced sensitive method for examining renal perfusion non-invasively, which we are still pursuing (Figure 2).
6) Moreover, we have identified the role of endothelial dysfunction in post-burn trauma, and suggest that oral fluids can be protective. Specifically, the presence of the glycocalyx in the kidneys has been shown with Syndecan-1 western blotting of renal tissue. This can also be localized with histochemistry to various lectins (Figure 3) and initial data indicates thay ORS is able to protect the glycocalyx.

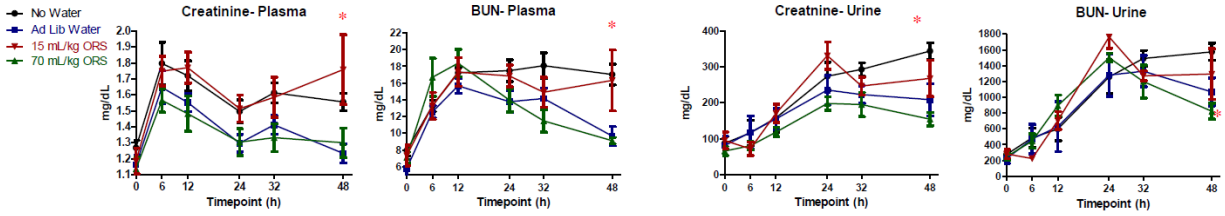

Figure 1. Initial increases in creatinine and BUN indicate significant AKI occurs early post-burn. Access to drinking water and/or volume-matched ORS reduces creatinine and BUN both in the plasma (left panels) and the urine (right panels). This occurs significantly compared to animals receiving water deprivation, or lower amounts of ORS (*-P<0.05, **-P<0.01).

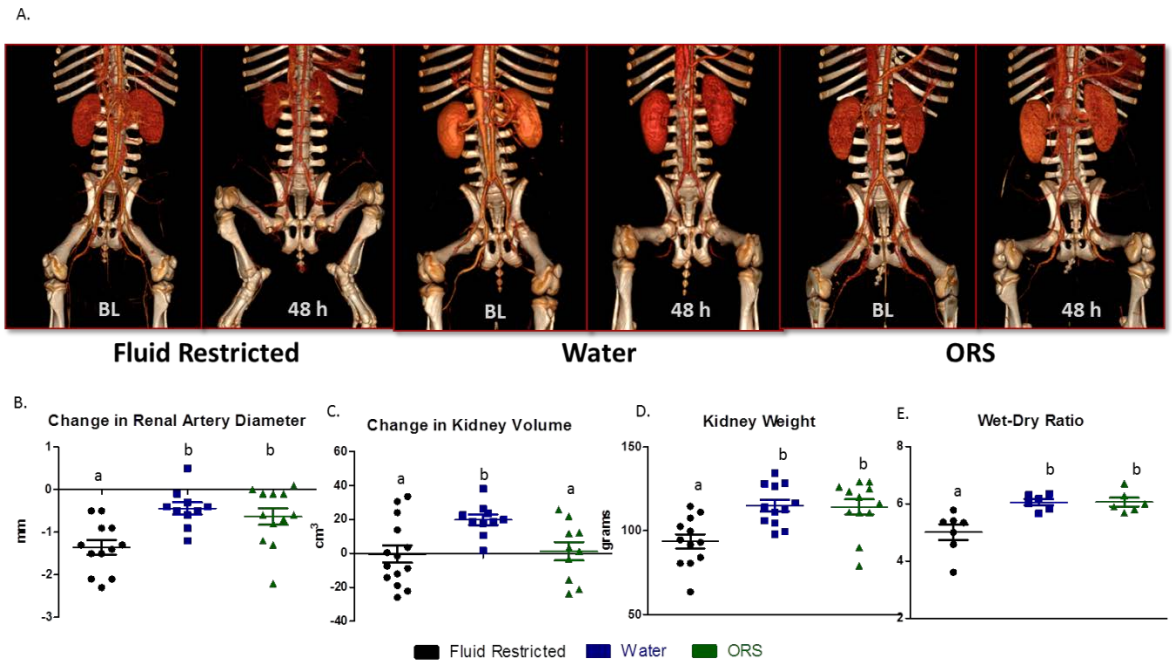

Figure 2. Computed tomography (CT) scanning (A) was performed pre-injury and immediately prior to euthanasia (termination of experiment 48 h). Renal artery diameter (B), kidney volume (C), weight (D), and wet:dry ratios (E) were quantified. For all parameters measured, changes from baseline to 48 h post burn are represented as mean ± SEM. Groups with different superscripts are significantly different ($P < 0.05$).

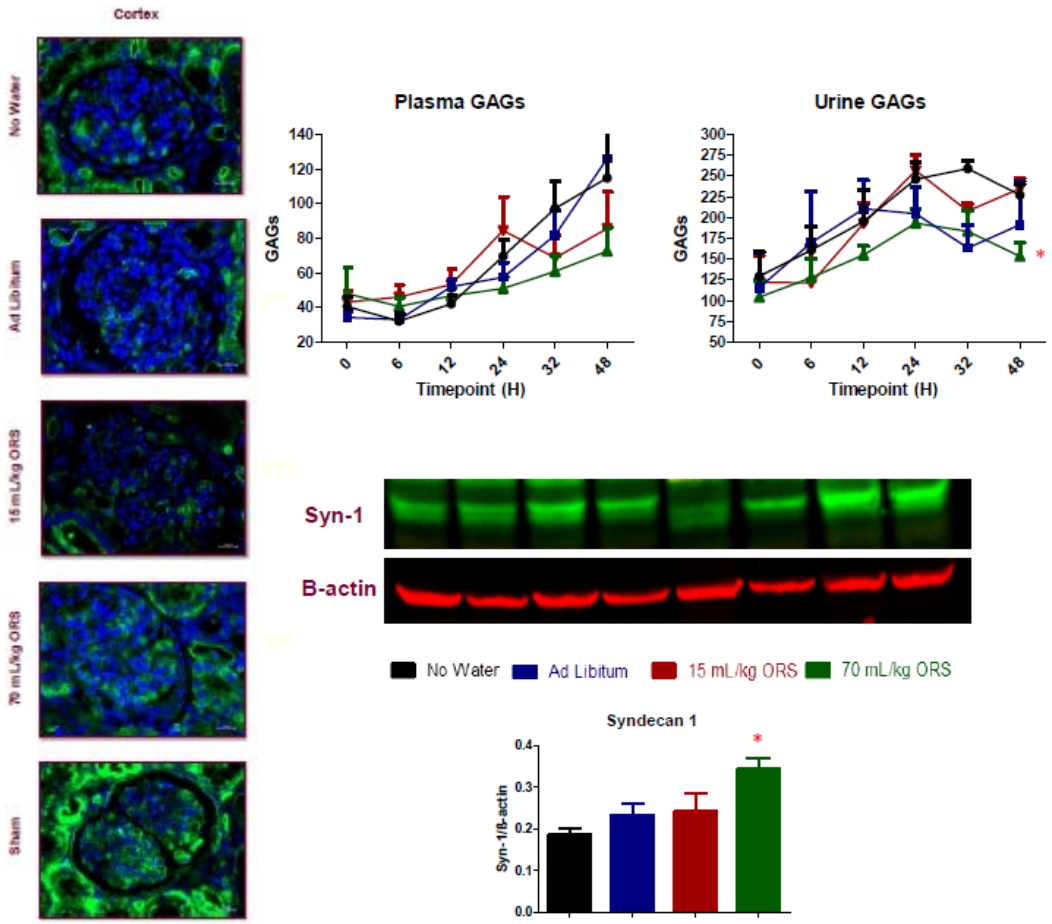

Figure 3. Left panel shows lectin staining in the glomerulus of kidneys, and indicates that the higher ORS group contains more glycosaminoglycans within the kidney. Consistently, the top right panels show less shedding of glycosaminoglycans both in the plasma and the urine. Finally, Western blotting of the kidneys reveals the highest syndecan-1 expression in the kidneys of animals receiving the high level of ORS.

Stated goals not met: Identify the effect of gastroenteral resuscitation on systemic and local inflammation.

Many of the ELISA analyses of circulating cytokines have been performed, and local renal levels need to be examined, which should occur in the very near future.

Taken together, these results indicate that enteral fluids may be a viable option for resuscitation of burn patients. From a safety standpoint, there were no adverse events (e.g., ileus) in our animal experiments, and these fluids were able to reverse acute kidney injury seen after 40% TBSA contact burn. While this method of delivering fluids is very simple and feasible in prolonged field care scenarios, it also may prove to be of benefit along with intravenous fluids as part of definitive clinical care. As such, one of the most exciting accomplishments emanating from this conclusion is the initiation of dialogue with the burn center at USAISR for a pilot study examining if this strategy is useful in patients as well. The best way to proceed with identifying the potential efficacy 0of enteral resuscitation in the clinic is currently being discussed, with data generation likely to occur in FY18.

What opportunities for training and professional development has the project provided?
While this project was not intended to specifically provide training or professional development, it has served as a platform for Dr. Burmeister to establish his laboratory, to include mentorship of two postdoctoral fellows who are involved with this project. This includes design of *ex vivo* experiments and writing/presentation of abstracts. Specific presentations have been done at national conferences including Shock, Experimental Biology, American Burn Association, and Military Health Research Symposium.

How were the results disseminated to communities of interest?
Nothing to report (manuscript preparation in progress).

What do you plan to do during the next reporting period to accomplish the goals?
The next reporting period will include: 1- Continuation of Animal Experiments, 2- Histological analyses, 3- Analysis of protein levels, and 4- Manuscript submission

IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
The finding and results from Year 1 of this project indicate that enteral fluids may be of benefit, and it is anticipated that studies into the types and volumes of these fluids will be pursued. Ultimately, this strategy may eventually be employed routinely for the resuscitation of burn patients.

What was the impact on other disciplines?
Nothing to Report.

What was the impact on technology transfer?
As mentioned earlier, these results have been communicated to the burn center. Discussions with Dr. Cancio at the USAISR burn center have begun with the intent of examining whether enteral resuscitation as a strategy should be adopted into practice. The best way to generate pilot data indicating whether this is worthwhile is currently under discussion.

What was the impact on society beyond science and technology?
If the above pilot data proves to corroborate the animal studies performed in Year 1, then it is possible that burn centers across the country/world will employ the use of enteral fluids. This would have far-reaching effects, especially in economically disadvantaged areas of the world.

CHANGES/PROBLEMS:
Changes in approach and reasons for change
Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
In Q1, contracting problems with the veterinary staff prevented a swine shipment on November 17, 2016 (see attached MFR). However, because our animal protocol was approved ahead of schedule, this did not impede our progress as stated in the SoW.

Changes that had a significant impact on expenditures
Hiring of a technician look a little longer than anticipated (hired in March 2017). If this individual is not able to finish the processing of all tissues at the end of FY18, a no-cost extension may be needed.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report.

PRODUCTS:
List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

Journal publications.
Burmeister DM, Gomez B, Dubick MA. Molecular mechanisms of trauma-induced Acute Kidney Injury: Inflammatory and metabolic insights from animal models. Biochim Biophys Acta; 2017 Apr 19; doi: awaiting publication. Yes.

Books or other non-periodical, one-time publications.
Nothing to Report.

Other publications, conference papers, and presentations.
Three abstracts were also published in conference proceedings:

1. Belinda I. Gómez, Matthew K. McIntyre, Michael A. Dubick, David M. Burmeister. Enteral Resuscitation with WHO Oral Rehydration Salts Ameliorates Burn-Induced Kidney Injury (AKI) and is Protective of the Glomerular Glycocalyx in a Swine Model. The FASEB Journal. Apr 2017; 31:S1030.14.
2. David M. Burmeister, Matthew K. McIntyre, Belinda I. Gomez, Robbie Montgomery, and Michael A. Dubick. The Growth and Differentiation Capacity of Porcine Renal Papilla Progenitor Cells Decline with Passaging. The FASEB Journal Apr 2017; 31(1): 731.4.
3. Belinda I. Gómez, PhD, Tony Chao, PhD, Matthew. K. McIntyre BA, Joshua S. Little, SPC, Michael A. Dubick, PhD, David M. Burmeister, PhD. Resuscitation with Oral Rehydration Salts Improves MOD Biomarkers in a Pig Burn Model. The Shock Society, Jun 2017.

Two abstracts were presented at the 2017 Military Health System Research Symposium, the first as an oral presentation, and the second received 3rd place in the poster competition.

1. Tony Chao, Ph.D., Belinda Gómez, Ph.D., Tiffany Heard, SPC Joshua Little, Michael Dubick, Ph.D., David Burmeister, Ph.D. Altered Mitochondrial Activity in Lymphocytes in Severely Burned Swine. Military Health System Research Symposium, Kissimmee, FL, August 2017.
2. B. I. Gómez, PhD, T. Chao, PhD, J. S. Little, PFC, M. K. McIntyre BS, M. A. Dubick, PhD, D. M. Burmeister, PhD. Fluid Deprivation Exacerbates Renal Dysfunction in a

Porcine 40% TBSA Burn Model. Military Health System Research Symposium, Kissimmee, FL, August 2017.

- **Website(s) or other Internet site(s)**
  Nothing to Report.

- **Technologies or techniques**
  Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Nothing to Report.

- **Other Products**
  Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	David Burmeister
Project Role:	PI
Nearest person month worked:	12
Contribution to Project:	Dr. Burmeister is providing technical oversight and leadership of the protocol. Specifically, he will oversee regulatory approval, supervise data collection and analysis, and coordinate team meetings to review planning and execution of the study.
Name:	Belinda Gomez
Project Role:	Postdoctoral Fellow
Nearest person month worked:	10
Contribution to Project:	Dr. Gomez assisted with animal procedures and processed blood/tissue samples.
Name:	Tony Chao
Project Role:	Postdoctoral Fellow
Nearest person month worked:	5
Contribution to Project:	Dr. Chao assisted with animal procedures and processed blood/tissue samples.
Name:	Tiffany Heard
Project Role:	Research Lab Technician III
Nearest person month worked:	7.0
Contribution to Project:	Tiffany is learning assays that examine mitochondria function.
Name:	Joshua Little
Project Role:	Private First Class
Nearest person month worked:	6
Contribution to Project:	Upon availability, PFC Little runs blood Vacutainer tubes to our biochemistry core lab, and aliquots plasma for later analysis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report.

SPECIAL REPORTING REQUIREMENTS
Quad charts: Attached

APPENDICES
Burmeister_BBA.pdf

BBA - Molecular Basis of Disease xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Molecular mechanisms of trauma-induced acute kidney injury: Inflammatory and metabolic insights from animal models☆

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ARTICLE INFO

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Animal models
Hemorrhage
Burn
Inflammation
Oxidative stress

ABSTRACT

Trauma-induced acute kidney injury (AKI), such as after hemorrhagic shock (HS) or burn, remains a significant problem in the intensive care unit and is associated with increased mortality. The pathophysiology that drives AKI post-trauma is multi-factorial, and includes both inflammatory and metabolic alterations. Identifying the systemic profile that contributes to AKI is crucial not only for early diagnosis, but also for identifying treatments that improve kidney function and maintaining long-term patient health. In an effort to elucidate this molecular pathophysiology researchers have utilized a variety of animal models including chemically-induced (i.e., cisplatin), blocking renal perfusion (i.e., arterial clamping) and inducing burn or HS. As the latter burn and HS models are unequivocally applicable to studying AKI in the context of traumatic injury, this review will summarize the inflammatory and metabolic insights associated with AKI gained with these animal models. Moreover, novel therapeutic strategies brought forth with these models will be discussed.

1. Introduction

Traumatic injuries such as hemorrhagic shock (HS) and burns can elicit a systemic cascade of metabolic and immunologic alterations that negatively affect distal organs [1]. The kidney is no exception, and HS or burn-induced acute kidney injury (AKI) is a common abrupt deterioration in kidney function that remains a significant problem in the intensive care unit, and complicates the prognosis of patients. This reduction in kidney function not only presents with immediate consequences during the initial hospitalization, but also leads to increased mortality and likelihood of progression to chronic kidney failure [2]. Over one quarter of patients with > 10% total body surface area (TBSA) burns develop AKI [3,4], and numerous studies in burn patients have shown that the presence of AKI post-burn is associated with significantly higher mortality compared to burn patients without AKI [3,5–8]. For example, a recent study showed patients with > 20% TBSA burns have a mortality rate of 19.9% which triples (62.4%) in patients who develop AKI [2]. Similarly, in cohorts of critically ill patients with various traumatic injuries including HS, diagnosis with AKI was associated with significantly higher in hospital mortality [9].

The pathophysiology that drives HS and burn-induced AKI is multifactorial and is not well understood. Thus, identifying the systemic alterations that contribute to AKI is crucial for early diagnosis, maintaining patient health and long-term kidney function. Many unanswered questions remain about the complex physiological response to trauma that propagates AKI in patients; therefore animal models are vital because they provide the ability to standardize the injury and collect multi-disciplinary data that address potential mechanisms involved. The purpose of this review is to provide a comprehensive overview of what animal models have taught us about the immunologic and metabolic physiological events that drive AKI in HS and burn trauma. This includes a discussion on the history of defining AKI in

Abbreviations: HS, hemorrhagic shock; AKI, acute kidney injury; TBSA, total body surface area; GFR, glomerular filtration rate; BUN, blood urea nitrogen; RIFLE, Risk, Injury, Failure, Loss of kidney function, End-stage kidney disease; ADQI, Acute Dialysis Quality Initiative; AKIN, Acute Kidney Injury Network; KDIGO, Kidney Disease Improving Global Outcomes; MOD, multiple organ dysfunction; IL, Interleukin; MCP1, monocyte chemoattractant protein 1; MAP, mean arterial pressure; HIF, hypoxic inducible factor; VEGF, vascular endothelial growth factor; ROS, reactive oxygen species; HO, heme oxygenase; KO, knockout; RNS, reactive nitrogen species; SOD, superoxide dismutase; NO, nitric oxide; NFκB, nuclear factor κ-B; TNF-α, tumor necrosis factor-α; AQP, aquaporin; GSH, glutathione; MDA, malondialdehyde; TBARS, thiobarbituric acid-reactive substances; MPO, myeloperoxidase; LPS, lipopolysaccharide; TLR, toll-like receptor; TRAIL, TNF-related apoptosis-inducing ligand; DAMP, damage-associated molecular patterns; PAMP, pathogen-associated molecular patterns; HMGB1, high-mobility group box 1; MAPK, mitogen-associated protein kinase; Akt, protein kinase B; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal kinase; NOX, NADPH oxidase; EPO, erythropoietin; IKK, Iκβ kinase; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; MPTP, mitochondrial permeability transition pore; MSC, mesenchymal stem cell; CsA, cyclosporine A

☆ The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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trauma, as well as an analysis of the appropriateness of available AKI animal models. Finally, we include a discussion of promising therapies for the prevention and treatment of HS and burn-induced AKI.

2. AKI and trauma

While reduced glomerular filtration rates (GFR) present unequivocal evidence of renal dysfunction a number of surrogates exist, including novel potential biomarkers [10]. Traditionally, the clinical standards for diagnosis of AKI have been serum creatinine and blood urea nitrogen (BUN), however this has progressed to more sophisticated scoring systems that take into account urine output [11,12]. AKI has also been referred to as acute renal failure and has been difficult to quantify because of the multiple definitions of AKI found in the literature. In the last decade, clearer standardized scoring systems have been implemented to provide a foundation for consistency in all studies, whether investigational/animal, observational, retrospective, or prospective. One of these classification schema termed RIFLE (acronym for: risk of kidney dysfunction, injury to the kidney, failure of kidney function, loss of kidney function, and end-stage kidney disease) was the first attempt by the Acute Dialysis Quality Initiative (ADQI) to standardize AKI [13]. Additionally, the Acute Kidney Network (AKIN) modified the RIFLE system into three AKIN stages to define AKI more strategically [14]. Specifically, while both of these definitions utilize serum creatinine and/or urine output, the AKIN definition allows for the incorporation of small rises in serum creatinine if they occur within a 48 hour period.

More recently in 2012 these scoring systems of acute kidney dysfunction were combined by Kidney Disease Improving Global Outcomes (KDIGO) group [15]. The severity of AKI according to KDIGO classification is diagnosed by an AKI staging system that distinguishes the state of injury based upon the rise in serum creatinine and fall in urine output. Three criteria can be used to diagnose AKI stage I: 1) serum creatinine levels rise at least 0.3 mg/dL, 2) creatinine increases 1.5 to 1.9 times from the initial measurement or 3) urine output is < 0.5 mL/kg/h for 6-12 h. Stage II requires elevated serum creatinine between 2.0 and 2.9 times from baseline or urine output is < 0.5 mL/kg/h for 12 h or greater. Finally, stage III is characterized by an increase in serum creatinine ≥ 3.0 times from baseline, or ≥ 4.0 mg/dL, anuria for $>$ 12 h, or a urine output < 0.3 mL/kg/h for > 24 h. While the criteria that dictate the presence of AKI in these different classification systems are very similar, their use may be tailored depending on the indication. For example, in critically ill patients, the KDIGO criteria was shown to be more sensitive in identifying AKI than the other two systems, and was more predictive of in-hospital mortality than RIFLE criteria, but not AKIN criteria [16].

In the thermally-injured patient, AKI may be classified as “early” or “late” based on whether the time to onset was before or after day ~5. Early-onset AKI usually relates to the initial reduction in plasma volume (i.e., hypovolemia) which decreases tissue perfusion [17,18]. The resultant ischemia/hypoxia has a synergistic effect with systemic inflammation and manifests in several physiological impairments (hyperglycemia, elevated body temperature, muscle wasting, etc.) because of a generalized hypermetabolic state [19]. Late-onset AKI is often driven by sepsis or associated with multiple organ dysfunction (MOD). Left untreated, systemic inflammation is often associated with a cytokine storm and oxidant burst, and is a major determinant in the development of MOD that often produces lethal results [20]. Similar to early AKI in thermally injured patients, HS patients experience a reduction in cardiac output that leads to poor organ perfusion, and hypoxia as a result of an immediate loss in blood volume. In this regard, HS results in even more extreme hypovolemia compared to burns, which is also coupled with metabolic and immunologic alterations to provide the foundation for the onset of AKI.

Severe traumatic injuries result in an influx of pro-inflammatory markers (e.g., cytokines, other signaling molecules) that have the potential to supplement clinical data to predict AKI and/or patient outcomes [21–23]. In trauma patients who initially survive their injuries, increased plasma levels of the cytokines interleukin-1 (IL-1), IL-6, IL-8, and monocyte chemoattractant protein 1 (MCP1) within the first 24 h of admittance were associated with AKI occurrence [24]. If prolonged, these elevated levels of pro-inflammatory cytokines contribute to muscle cachexia and elevated whole body protein turnover in thermally-injured patients [25]. Particularly for burn trauma, the associated hypermetabolism further exacerbates kidney dysfunction, which can be lethal if essential protein reserves are severely depleted [26]. This review will summarize the intertwined nature of the relationship between oxidative stress markers and the innate immune response to both HS and burn injury.

3. Animal trauma models studying AKI

Although, the impact of AKI varies based on trauma severity and other co-morbidities, identifying and understanding the molecular abnormalities responsible for this condition is vital for early diagnosis and treatment. In an effort to understand the post-traumatic molecular mechanisms behind AKI, researchers have employed a variety of animal models of traumatic injury. Generating standardized animal models to mimic a clinically relevant situation of burn or hemorrhagic shock is difficult. In fact, some have argued that the more researchers control variables in their models, the less clinically translatable the data become [27,28]. For example, in trying to understand the pathophysiology associated with reduced kidney blood flow many studies incorporate clamping of the renal artery which occludes blood flow completely. These animal models have been reviewed elsewhere, but may not induce the systemic alteration seen in HS trauma patients [29]. Uncontrolled HS animal models may more closely resemble the clinical setting however, reproducibility of the model may be challenging [27,30].

Similarly, while animal models of mechanical and chemical (e.g., cisplatin) induced AKI may afford limited insights into burn-induced early onset and late-onset AKI, respectively, neither recapitulates the complex pathophysiology of burn injury. In addition, one of the leading causes of AKI in critically ill patients is the potential for sepsis, and animal models of sepsis-induced AKI (e.g., cecal ligation and puncture) may reproduce only certain aspects of burn-associated AKI [31]. Also, the extra endotoxin stimulus of LPS/sepsis models may confound the molecular basis for AKI after burn alone. Taken together, the models discussed above will be referenced sparingly, with a focus on animal models of AKI due to burn or HS, and the inflammatory/metabolic insights generated with them.

Articles for this review were selected using two independent searches, one for HS and the other for burn. Criteria for including articles into this review after both searches can be seen in Fig. 1. The first literature search in PubMed using “acute kidney injury” and “hemorrhage” with "Other Animals" checked turned up 2182 results as of October 18, 2016. As discussed above, several of the articles excluded from this review utilized a model of kidney damage by clamping the renal artery to focus primarily on the damaging effects of reperfusion. Traditionally, animal models of HS incorporate fixed pressure, fixed volume or uncontrolled hemorrhage, with or without a secondary traumatic insult. In this PubMed search, models of HS primarily included fixed pressure, where mean arterial pressure (MAP) was maintained between 30 and 45 mm Hg for a period between 30 and 180 min in anesthetized female or male mice and rats. Following HS, animals are often resuscitated with their shed blood, lactated Ringer's, or normal saline. The most widely employed model is the rodent, while others have incorporated swine, dogs, and to a lesser extent rabbits and sheep. Swine models were primarily used for evaluating hemorrhage control and resuscitation [32–34] and rodent models for understanding signal transduction mechanisms. Additionally, rodent models were often utilized to screen various treatments to reduce the incidence or severity of

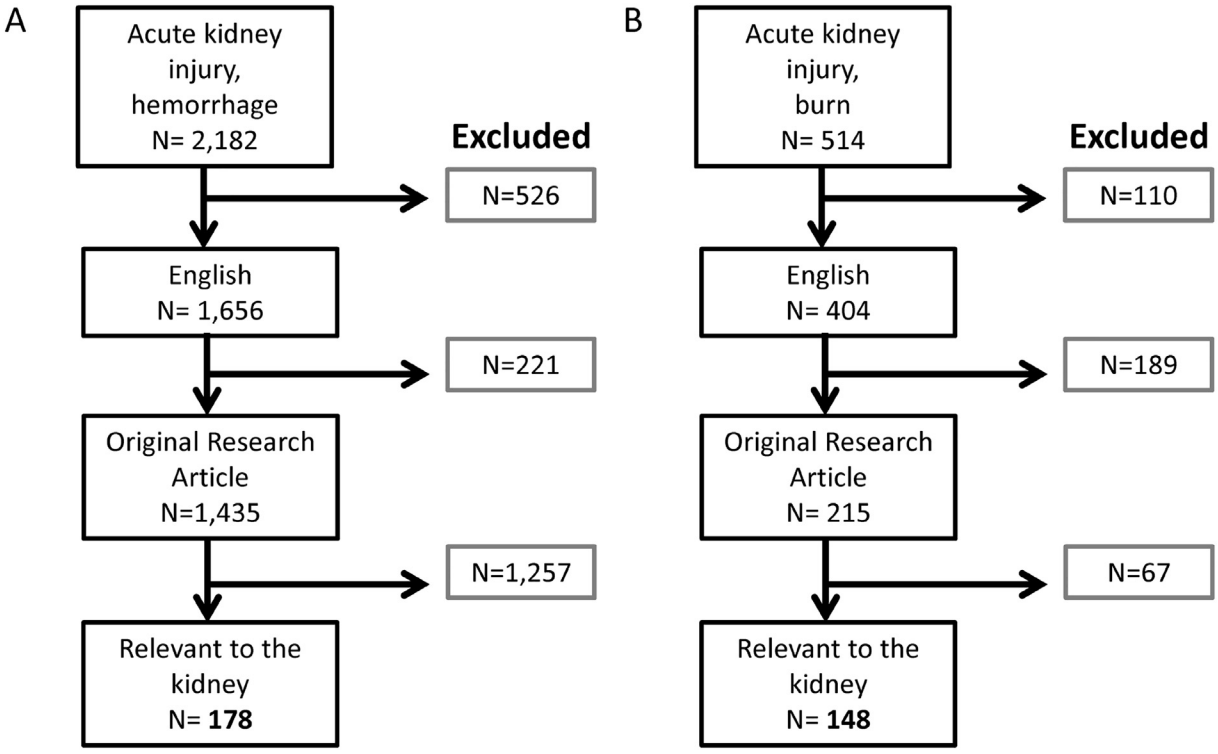

Fig. 1. Criteria for articles included in review from two individual PubMed literature searches using the key words: A) "acute kidney injury, hemorrhage" for retrieval of articles for hemorrhagic shock and B) "acute kidney injury, burn" for articles on burn injury.

AKI.

Animal models of burn-induced AKI have been utilized since the 1970s and initially were largely descriptive about renal morphology and cell proliferation [35–38]. To acquire articles for AKI in thermally-injured animals, "acute kidney injury" and "burn" were entered as search criteria into PubMed with 514 results as of October 26, 2016. Exclusion criteria for these studies are also shown in Fig. 1. The overwhelming majority of animal burn models reviewed herein incorporated a scald injury in the range of 20–40% TBSA on the dorsum of mice or rats. Following the immersion of the animal in boiling water for a few seconds, animals are often resuscitated intraperitoneally. In general, large animal burn models are regarded as superior clinical surrogates for burn injuries [39]. However, similar to HS, rodent models are usually utilized to examine molecular pathophysiology, likely due to the available tools (e.g., antibodies, knockout animals) available for the studies.

The type of evidence that investigators utilize to illustrate successful onset of AKI also varies widely. While KDIGO criteria of AKI are now routinely used in patients, they have not been widely applied in animal models of AKI. Using KDIGO criteria may be problematic because of varying normal levels of creatinine and urine output depending on the species [40]. For example, in rats baseline creatinine is normally around 0.3 mg/dL. Therefore, an increase of 0.3 mg/dL (which would be KDIGO stage 1 in humans) would actually constitute a doubling (i.e., KDIGO stage 2). Another limitation is that many of the animal studies are too short-term for the human scoring systems to be applied. Therefore, appropriately scaled values should be used for applying information used from animal models to the human condition to better link the animal studies with severity scores of human AKI. This strategy has recently been employed by us for defining systemic inflammatory response syndrome in swine to account for their increased body temperature and white blood cells counts under healthy conditions [41]. This also highlights an advantage of animal models in that baseline (pre-trauma) values can be obtained, which is almost never the case clinically.

Thus, when addressing the validity of animal models of AKI, the criteria used to define it are essential. For example, many studies describe the onset of AKI from a purely histopathological standpoint or a measurement of blood flow. The immunological and metabolic alterations in response to hypoxia contribute to the damage within the microvascular and tubular components of the kidney, which can be seen histologically. In one study following HS, several blood-filled and shrunken glomeruli were present and tubular necrosis was demonstrated by detached basal lamina [42]. Similarly, while models of electrical burns (and associated rhabdomyolysis) may be of benefit in studying AKI, only histological changes (glomerular congestion, proximal tubule degeneration) were shown without evidencing any functional changes [43]. This microstructural damage is not indicative of decreased renal function per se, and AKI can also occur in the absence of significant structural changes seen histopathologically [44]. Also, in the case of sepsis-induced AKI, renal dysfunction can occur concomitantly with normal, or even increased, blood flow, and global blood flow to the kidney may not be as important as localized areas of impaired perfusion [31]. As such, the overwhelming majority of the animal studies included in this review had creatinine and/or urine output data available.

The sections below summarize the information learned from animal studies on the mechanistic basis of HS and burn-induced AKI. Individual examples in burn models and HS models are given and are discussed together because of the significant overlap between inflammatory and metabolic responses in the two trauma subtypes. However, there are also instances of unique molecules in either scenario that will be noted. Many of these molecular players are summarized graphically in Fig. 2. This figure provides a unifying picture and will be referred to often, as it also depicts which signals are shared or unique to burn or HS injuries.

4. Oxidative stress in AKI

The signaling cascade that compromises kidney function in HS animals is initiated primarily by hypoperfusion resulting from the drop in

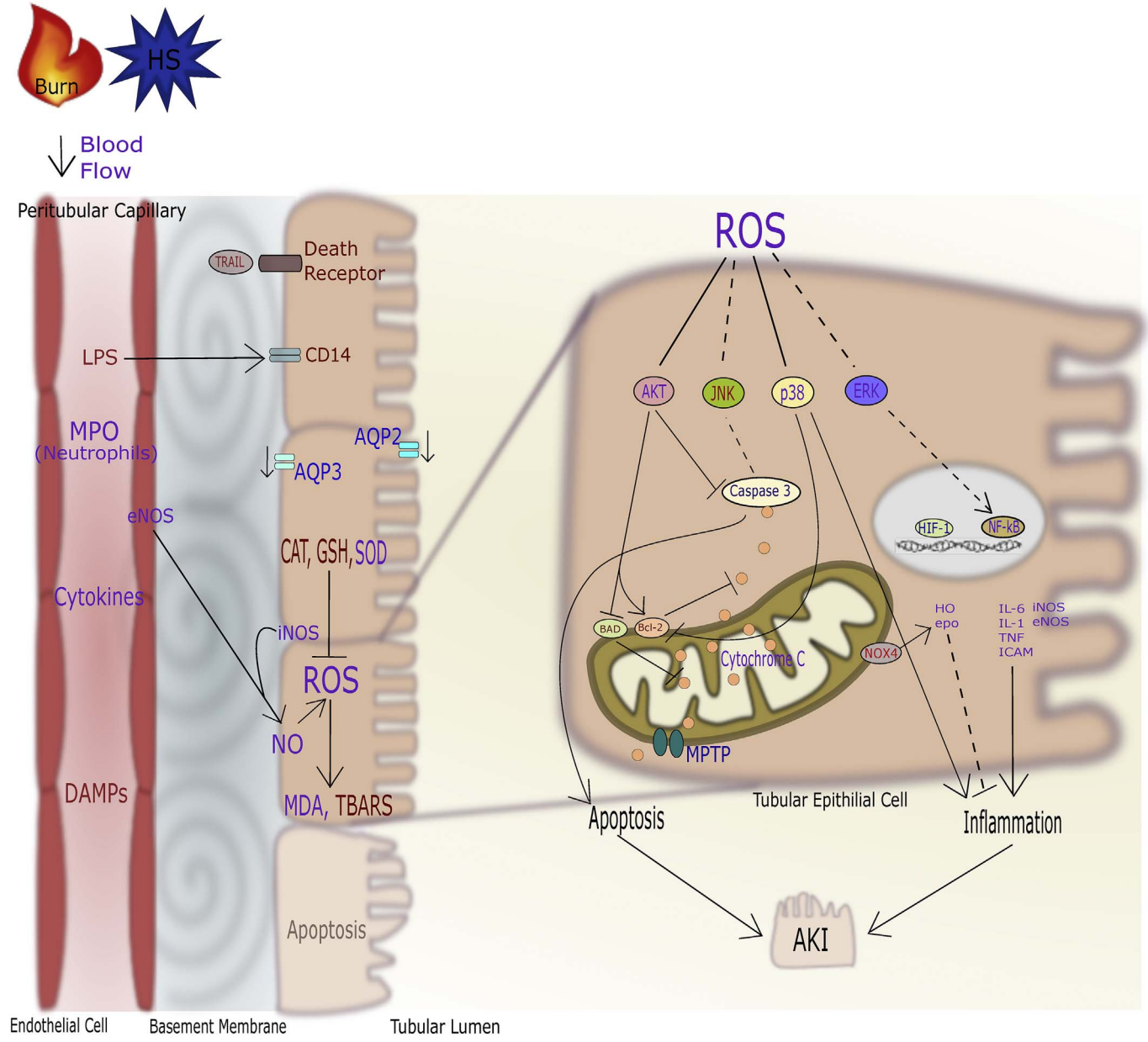

Fig. 2. Burn or hemorrhagic shock (HS) both result in reduced renal perfusion [17,18,60] that generates inflammatory and metabolic alterations in the tubule epithelia associated with acute kidney injury (AKI). Traumatic injury elicits an associated accumulation of reactive oxygen species (ROS) that result in activation of mitogen-activated protein kinases (MAPK) families such as c-Jun N-terminal kinase (JNK), p38, and, perhaps in burns, extracellular signal-regulated kinase (ERK) [75,120]. The antioxidant enzymes superoxide dismutase (SOD) [58,68], catalase (CAT) [73], and glutathione (GSH) [74] are also reduced resulting in further ROS accumulation and increased malondialdehyde (MDA) [58,74] and thiobarbituric acid (TBARS) levels. In burns, activation of the MAPK such as p38 enhances the expression of inflammatory cytokines [108,120] and induces apoptosis. Alternatively, activation of Akt positively regulates the promoter of cell survival, B-cell lymphoma 2 (Bcl-2), and negatively regulates the pro-apoptotic protein Bcl-2-associated death promoter (Bad). The result is the release of cytochrome C which is crucial because of the association with caspase and subsequent apoptosis [123]. In burn, cell death is also mediated by TNF-related apoptosis-inducing ligand (TRAIL) and death receptor 5 [90] and inflammation is, in part, driven by elevated CD14 in the absence of infection [85]. In HS, this may be mediated by opening of the mitochondrial permeability transition pore (MPTP) [60]. In both types of trauma, nuclear factor κ-B (NFκB) is translocated to the nucleus and initiates the transcription of target genes that include interleukin 6 (IL-6) [58], IL-1 [24], tumor necrosis factor α (TNF-α) [58,141], and ICAM [58,75]. In HS, NFκB also upregulates expression of nitric oxide synthases (NOS) that further exacerbates cellular oxidative stress [49,58]. In addition, hypoxic inducible factor (HIF) is stabilized and activated for the transcription of heme oxygenase (HO) [49,51], which catabolizes free heme [54]. Proximal tubule cells in HS are further compromised by disrupted water balance following reduced aquaporin 2 and 3 expression [64]. Finally, the role of damage-associated molecular pattern (DAMPs) in trauma is just being explored, and may reveal novel molecular targets [142]. Molecules in blue demonstrate regulation with HS, red denotes changes induced by burn, and purple denotes involvement in both HS and burn. Dotted lines indicate conflicting or minimal evidence is currently available.

blood pressure, and limited oxygen availability to the outer medulla. A minor fall in arterial blood pressure in a normally functioning kidney would elicit intrinsic signaling pathways (e.g., efferent arteriole constriction) to prevent adverse reductions in GFR. This autoregulation is an essential feedback mechanism to keep the glomerular filtration rate constant across a wide range of arterial blood pressures. However, the hypoperfusion that occurs after a major fall in MAP (i.e., < 50 mm Hg) stimulates several systemic and intrinsic mediators within the kidney. Indeed, this may constitute the major difference between HS and burn injuries, as burn-induced AKI may occur in the absence of hypovolemia/hypoperfusion. Infusion of plasma from burned rats into healthy animals causes leukocyte activation and enhanced fluid extravasation and edema [45]. While the burn eschar itself may be a nidus for inflammatory substances, this study indicates that it is not necessary for the induction of systemic inflammation. Alternatively, others have postulated that the initial damage to the intestine may be the source of

cytokines that damage distant organs [46].

Generally, in response to low oxygen tension, cells target to stabilize and activate hypoxic inducible factors (HIFs), a heterodimer (HIF-α and HIF-β subunits) which regulate transcriptional activity to coordinate the adaptive response to hypoxia [47]. In renal artery clamp models HIF1-α protein was induced within an hour post-insult in whole kidneys of pigs, and collecting ducts, papillary tubular cells and interstitial cells of rats [47,48]. However, in HS mice mRNA expression of HIF-α, was not different from sham suggesting that HIF-α promptly responds to hypoxia by post-translational modifications [49,50]. These studies implicating hypoxia via increased levels of HIF within the kidney have not been reported in burn injury. While (especially from an inflammation standpoint) there are definitive examples of molecular overlap between HS and burn injuries (Fig. 2), the differences in hypoperfusion likely results in distinct targets in the two injury patterns.

Target genes of HIFs are the vascular endothelial growth factors (VEGFs), a family of signaling proteins that promote angiogenesis and vascular maintenance. In HS rats (MAP maintained at 35 ± 5 mm Hg) and pigs (MAP maintained at 40 mm Hg), kidney expression of these growth factors and their receptors is reduced however; lack of expression may be due to time of collection or severity of kidney injury [49,51]. Interestingly, in diabetic HS mice VEGF-2 receptor was reduced to a lesser extent than in non-diabetic HS mice [49]. These authors further suggested HS effects on the kidney were more severe in mice with type II diabetes as diabetic HS mice displayed elevated creatinine, medullary cellular fragmentation, and vacuolization, while they failed to induce AKI in non-diabetic HS mice (i.e., creatinine did not change). To date we did not find literature on expression of these receptors in the kidneys of thermally-injured animals yet, researchers have studied the effects of VEGF and other growth factors on burn wound healing [52].

Ischemia following hemorrhagic shock has resulted in free heme which contributes to the production of reactive oxygen species (ROS) and oxidative stress within the kidney [53]. As shown in Fig. 2, ROS have been reported to be an upstream mediator of many molecular responses in both burn and HS. To counteract the accumulation of ROS, another target of HIF is heme oxygenase (HO), an enzyme that protects cells from free heme through oxidative catabolism. In a rat HS model HO-1 mRNA expression in tubular epithelial cells was rapidly induced within 3 h following HS and reperfusion [54,55]. In HO-1 knockout (KO) mice caspase 3 was robustly increased, and kidney damage was apparent with renal artery clamping for 15 min [56]. Kidney damage was assessed by significantly elevated creatinine in HO-1 KO injured mice compared with shams. HO-1 expression (and serum creatinine) has also been shown to be increased within the kidneys of mice after 15% TBSA burn injury [57].

The generation of reactive oxygen and nitrogen species (ROS and RNS, respectively) has been associated with HS-induced renal dysfunction. Renal malondialdehyde (MDA) generation, reduction in superoxide dismutase (SOD) activity and induction of inducible nitric oxide synthase (iNOS) and subsequent nitric oxide (NO) production were shown after HS in rats [42]. ROS and NOS facilitate the translocation of the transcription factor nuclear factor κ-B (NF-κB) from the cytoplasm to the nucleus, which elicits the inflammatory cascade. Once in the nucleus, NF-κB initiates expression of iNOS [49,51], tumor necrosis factor α (TNF-α) [58–60] and interleukin 6 (IL-6); all elevated in rat kidneys within 2 h of HS and resuscitation [42]. iNOS and NO production are also generated during hypoxia in rat proximal tubules [58,61]. NO derived from iNOS propagates the inflammatory response and contributes to enhanced tissue damage. Additionally, endothelial nitric oxide synthase (eNOS) was capable of generating NO in the kidney 6 h following HS in pigs [51] but not in mice [49]. In contrast, hypoxia from hemorrhage in rats was associated with a 10-fold drop in NO and renal damage and dysfunction [62]. NO has also been shown to regulate water permeability via inhibition of vasopressin [63]. Additionally, HS further reduced the abundance of aquaporins (AQP), a family of membrane protein water channels, in the kidney [64]. In principal cells of distal or collecting tubules, AQP2 in the luminal membrane and AQP3 in the basolateral membrane, but not AQP1 in the proximal tubule, was reduced following HS. However, whether NO acts via a direct mechanism in reducing water permeability through AQP has yet to be investigated. The status of renal aquaporins has been far less studied in burn animal models.

The kidney contains an abundance of polyunsaturated fatty acids, which makes it particularly vulnerable to oxidative stress regardless of trauma type. Numerous studies in experimental animals and humans have reported reduced antioxidant status related to thermal injury [65]. A rat model of extensive burn was utilized to provide initial evidence about the importance of free radicals in burn-induced AKI [66]. Since most free radicals have extremely short half-lives, luminol-mediated chemiluminescent reactions have been used as a proxy for ROS levels in the kidney [67]. In addition, it is commonplace to measure activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, as well as reduced glutathione (GSH) in kidney tissues post-burn and HS. MDA and thiobarbituric acid-reactive substances (TBARS) as markers of ROS-induced lipid peroxidation have also become routine measurements in animal models. All of these are indicated in Fig. 2.

As an example, Gotoh et al. utilized 2 different levels of burn injury (i.e., 35 and 60% TBSA) to demonstrate that while mRNA levels of SOD increased in the kidney in a dose-dependent fashion, the amount of SOD protein was not elevated dose-dependently [68]. In addition, a series of investigations by Sener et al. was among the first to examine the effects of antioxidant therapy on ROS markers and kidney function (i.e., creatinine) post-burn injury [69–73]. These studies utilized a full-thickness scald injury in rats to examine the benefits of anti-inflammatory compounds such as melatonin, gingko biloba extract, and Rosiglitazone after 24 h. These results, along with others [74], established very clearly that the amount of MDA in the kidney increases post-burn, while GSH levels concurrently decreased. While the antioxidants tested consistently reversed these findings, the effects on kidney function were not as clear. Some antioxidants exerted a marked beneficial effect on creatinine levels [72] compared to others [71]. Moreover, despite consistent methods, the creatinine levels were not always increased to the same degree by the injury [73], highlighting the substantial challenge of variability in animal models of AKI that inherently comes with in vivo studies. A similar study performed by Wang et al. demonstrated that the increased MDA levels after HS in rats was reduced by treatment with the antioxidant crocetin [58]. These researchers concluded that although HS reduced total SOD, crocetin provided protection by elevating total SOD. Administration of hydrogen sulfide also neutralized reactive molecules and has been shown to reduce kidney injury scores in a HS + ischemia reperfusion (aortic cross clamp) pig model [51]; however, creatinine levels were not affected by hydrogen sulfide treatment at the time of animal euthanasia (6 h) [51]. Clinically, it seems that while targeting free radicals may show some benefit, any successful treatment for end organ damage needs to exert pleiotropic effects or will require some type of treatment cocktail.

5. Immunologic alterations in AKI

Related to the discussion on oxidative stress on the kidney in burn and non-burn trauma, many studies have also implicated altered levels of myeloperoxidase (MPO) in the kidney (Fig. 2) [71–73,75–77]. This enzyme is abundantly released by the neutrophil during the cells' respiratory burst and an enzyme assay is used as a marker of neutrophil activity within tissue. MPO generates hypochlorous acid which neutralizes bacteria, but also may cause oxidative damage in the host tissue. Infection is a serious consideration in burn patients; however animal models have proven that neutrophil levels rise even in the absence of infection [41]. Additionally, MPO positive cells in the kidney medulla, but not cortex, are elevated by 5 h following HS [78].

While the above studies suggest that neutrophil infiltration may be

an attractive target for AKI, most of the evidence to date has been associative or conflicting. In an ischemia/reperfusion model, knocking out an E-selectin ligand CD147 (affecting extravasation), was used to show that neutrophil expression of CD147 was necessary for the development of AKI [79]. As mentioned earlier, a major limitation of this study was the sole use of BUN as a marker of renal function. In contrast, depleting neutrophils in an ischemia/reperfusion model was only shown to reduce serum creatinine marginally [80], more in line with earlier studies showing no change at all [81]. To our knowledge, studies in burn animal models that target neutrophil infiltration have not shown efficacy, perhaps due to the time-dependent nature of neutrophils within the kidney [82]. Again, the etiology of AKI is likely of the utmost importance, as neutrophil depletion was protective of renal function in sepsis- [83] but not cisplatin-induced AKI [84]. Clearly, more research needs to be done to elucidate whether targeting neutrophil activation in HS- or burn-induced AKI is a viable option.

The innate immune system has also been implicated in AKI in other ways. Another rat model of 35% TBSA scald injury has shown that thermal injury itself in the absence of infection can result in markedly increased levels of endotoxin in several organs including the kidney [85]. In this study, lipopolysaccharide (LPS) binding protein RNA was elevated in the kidney at 12 h after the injury. Moreover, the CD14 receptor (which binds LPS) was also elevated in the kidney for 2 days, further implicating the innate immune system. However, this study failed to show a significant increase in serum creatinine, making the applicability of CD14 and LPS to burn-induced AKI unclear. Also intriguing, is the possibility of implicating toll like receptor-4 (TLR-4), as initial CD14 binding of LPS ultimately presents the ligand to TLR-4 [86]. It is well known that TLR-4 is upregulated in the kidneys of septic animals [87], but its role in hemorrhage and/or burn-trauma remains largely unstudied.

In general, studies of inflammation associated with traumatic injury have often centered on plasma or tissue levels of cytokines (Fig. 2). Elevated levels post-burn or HS have often been implicated with contributing to the development of MOD, including AKI. For example, in a 35% full thickness rat burn model the cytokine tumor necrosis factor TNF-α and TNF-α receptor 1 (TNFR1) mRNA and protein levels were determined in kidney and other organs. Specifically, despite no changes in serum creatinine or renal levels of TNF-α mRNA, a strong correlation was observed between serum creatinine and TNF-αR1 [46]. The authors suggested that the expression of TNF and its receptor could be involved in the development of MOD following burns. This was further supported by a report studying early enteral feeding after a scald burn in rats, which significantly improved creatinine clearance, and reduced blood concentrations of TNF [88], despite no changes in circulating endotoxin. In addition, another study revealed that their 35% burn injury produced some perturbations in renal function, as evidenced by significantly increased BUN [89]. However, treatment of burn injury with a monoclonal antibody neutralizing TNF had no effect on BUN but did reduce markers of liver or muscle injury. Leng et al. have recently reported a critical role for the interaction between TNF-related apoptosis-inducing ligand (TRAIL) and death receptor 5 (DR5) in the pathogenesis of AKI. In their mouse burn model, serum creatinine and BUN progressively rose over 24 h, and administration of soluble DR5 reduced creatinine, as well as alleviated apoptosis and injury score in the kidney [90]. Taken together these studies implicate the TNF family of proteins in AKI after burn.

Other inflammatory players such as, damage-associated molecular pattern (DAMPs) and pathogen-associated molecular pattern (PAMPs) are of great interest for AKI as they are filtered by the kidney and can trigger inflammatory responses and bioenergetics failure, but their association with AKI has not been studied in burn or HS animal models in detail. DAMPs and PAMPs have been implicated often in sepsis-induced AKI [91], and therefore may have a role in late-onset AKI related to infection. A protein of specific interest, high-mobility group box 1 (HMGB1) has been shown to be unregulated in rat kidneys (as well as lung and liver) on both the mRNA and protein levels post-burn [91]; however this study made no attempt to determine AKI by creatinine or urine output.

As a critical regulator of the immune response that responds to both endogenous and exogenous patterns, the complement system is also a logical pathway to analyze. The complement system is implicated in AKI of various etiologies, which has been reviewed elsewhere [92]. While, to date, the evidence implicating the complement system in sepsis is more extensive than burn or HS, some studies may indicate pathway specificity. Importantly, the complement system is divided into the classical (C1), alternative (C3) and Lectin pathways. Previous studies in burn have utilized a 30% TBSA swine burn model to show that inhibition of C1 (classical pathway) prevents edema and bacterial translocation within the gut [93]. The same group showed C1 inhibition reduced capillary leakage and tubular degeneration within the kidneys [94]. Alternatively, C3 has been shown to be increased in burn wounds [95]. Kidney function was not assessed in these studies. However, it has been shown that in trauma patients, the alternative pathway predominates in the acute setting [96] and that the alternative pathway is responsible for tubular necrosis [97]. The alternative pathway has also been implicated in AKI in ischemia reperfusion models [97] which has also been reviewed elsewhere [98]. Despite this, inhibition of the classical pathway has been shown to reduce creatinine in a pig model of controlled hemorrhage [99]. In addition, in a swine hemorrhage model, marked renal tubular injury was observed that was less evident in animals treated with Decay Accelerating Factor, a classical and alternative pathway inhibitor [100]. Taken together, the information obtained from renal ischemia should be used to address the minimal data available on the role of the complement system in HS- and burn-induced AKI.

Altogether, while the effects of targeting one cytokine/DAMP/PAMP may be insufficient, it is also possible that non-specifically targeting all cytokines will not be advantageous as shown in a recent study of cytokine hemoadsorption in a swine burn and smoke inhalation model [101]. In that study, IL-1β, IL-6 and IL-10 were removed across the Cytosorb™ membrane, but led to no significant improvements on survival or inflammation. Moreover, the AKIN criteria was used to show stage 1 AKI, however hemoadsorption did not reduce creatinine levels. Also, lipid-mediated resolution of inflammation has been postulated in cases such as sepsis. For example, a recent study used a rat model of burn and sepsis-like conditions (LPS injection) to show the improvements in renal pathological changes due to resolvin D2 [102]. However, the authors did not show any significant increase in creatinine due to their injury. In short, the prospect of altering the levels of these inflammatory signals in the post-burn kidney warrants further investigation and identifying inflammatory markers for effective therapeutic targets to reduce AKI remains elusive.

6. Mitochondrial damage and apoptosis

Fig. 2 illustrates the importance and implication of renal mitochondria in AKI after traumatic injury. Mitochondria are the linchpin organelle essential for maintaining normal cell bioenergetics and are present in both microvascular endothelial cells and the highly energy-dependent kidney tubular cells. The above-referenced inflammatory responses elicit a disruption in mitochondrial function that includes a loss of ATP production and reduces the respiratory control rate of mitochondria in the kidney [60]. In a HS rat model based on respirometry (i.e., the ratio of mitochondrial oxygen consumption in the presence and absence of ADP), mitochondrial function was reported to be significantly reduced post HS [60]. However, additional studies have observed mitochondrial dysfunction associated with higher creatinine levels post HS (however no causation was determined) [103]. Recently, multiphoton microscopy was used to demonstrate damaged mitochondrial structure and reduced mitochondrial membrane potential after ischemia reperfusion [104]. While respirometry has been used to

explore dysfunction in other organs post-burn [105], the function and structure of renal mitochondria post-burn is less studied. Considering burn-induced hypermetabolism, the high energy needs of the kidney for solute transfer makes this organ particularly susceptible to mitochondrial damage.

Another aspect of HS and burn-induced AKI that has been of recent interest, relates to mitochondrial-induced apoptosis (Fig. 2) [106]. Certainly, the process of mitochondrial driven apoptosis is intimately tied in with oxidative stress and inflammation, and it is sometimes difficult to distinguish which processes are potentially causative. The molecular mechanisms behind mitochondria-related apoptosis seen post-trauma is being explored only recently. For example, the role of mitogen-associated protein kinases (MAPK) pathway such as p38 in mitochondrial apoptosis has been identified [107]. Implication of this MAPK pathway in regulation of inflammatory mediators in burn-induced AKI has also been shown [108]. These authors showed that an inhibitor of p38 MAPK reduced the rise in BUN seen post burn in rats, and ameliorated histological evidence of renal damage. Similarly in rats, treatment with FR167653, an inhibitor of p38 MAPK activation reduced mRNA expression of TNF-α, IL-1β, and prevented the expected increase in serum creatinine after HS [109]. More recently, several studies have examined this p38 pathway even further while simultaneously elucidating additional molecular mechanisms. For example, Guo et al. showed that serum creatinine levels tripled after 40% TBSA scald injury in rats, which was reduced with the anti-oxidant astaxanthin [110]. This anti-oxidant partially blocked lipid peroxidation (MDA) and restored levels of SOD and catalase, while preventing apoptosis. Cytochrome C, caspase 3, and caspase 9 were all significantly increased post-burn, and subsequently reduced with astaxanthin, suggesting reduction in mitochondrial damage. Molecularly, this was done by augmenting the protective phosphorylation of protein kinase B (Akt) and also increasing phosphorylation of bad (Bcl-2-associated death promoter homologue). As Fig. 2 illustrates, the bcl-2/bad axis of proteins is intimately involved with whether stressors results in cell survival or mitochondria-driven apoptosis [111], including in AKI [112].

It should be reiterated that many of the molecular players listed above are inseparable from the inflammatory molecules mentioned in the previous section. As Fig. 2 depicts, many of these signaling pathways are intertwined with one another. A prime example of this is the p38 MAPK pathway (along with other protein kinases), which not only respond to stress signals, but are also involved in cytokine production [113]. This remains true in several renal diseases, including AKI, wherein p38 inhibition prevents inflammation and apoptosis [114]. This has also been shown in a murine burn model, where topical application of p38 inhibitors reduces epithelial apoptosis and systemic inflammation [115,116]. New upstream targets are being identified that may act on multiple MAPKs for even more pronounced pleiotropic effects [117]. Certainly, more research into leveraging these pleiotropic effects for treating burn and/or HS-induced AKI is warranted.

The temporal aspects of these different molecular players should also be considered, as previous studies have shown that alterations of these key molecules in the kidney can last for several days [118,119]. In those studies, burn-induced AKI was associated with elevated renal concentrations of bcl-2 and major histocompatibility complex class I-chain antigen, which were sensitive to ulinistatin. While absolute levels of proteins like bcl-2 may be revealing, ultimately the phosphorylation status of these regulatory proteins represent their active form. In this regard, another study by Guo et al. confirmed burn-induced AKI in their rat model out to 72 h, and also further elucidated the mechanisms involving MAPKs [75]. Similarly, they found burn-induced alterations in serum creatinine, oxidative stress markers, apoptosis, and levels of cytokine expression. Importantly, time-dependent increases in the phosphorylation state of apoptosis-related MAPKs such as Akt, p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) was also shown. Moreover, there was an increase in the overall amount of the transcription factor NFκB, further linking these pathways to inflammation post-burn (Fig. 2). These changes were ameliorated (or augmented in the case of the anti-apoptotic signal Akt) with hydrogen-rich saline resuscitation [75]. Considering the ease of implementing this resuscitation fluid in practice, further research in this area should be considered.

Another study by Feng et al. investigated similar molecular mechanisms over the same 72 h time frame post-burn [120]. Increased creatinine, BUN, apoptosis (with caspase cleavage) and indices of oxidative stress (hydrogen peroxide and TBARS) were apparent over this time, as was a decrease in manganese-SOD (the mitochondrial-specific SOD isozyme). While this study found similar changes in P-Akt and P-p38, they did not find any differences in the phosphorylated JNK and ERK in contrast to the previous study. Despite these discrepancies, both studies implicated mitochondrial-driven apoptosis pathway in burn-induced AKI, and implied a potential therapeutic benefit from p38 MAPK inhibitors. Moreover, the Feng study [120] also found a 3-fold increase in NADPH oxidase 4 (NOX 4) expression in kidney tissue. This is an interesting finding, as NOX 4 has been postulated to be an “oxygen sensor” in renal mitochondria, and may lead to production of erythropoietin in the kidney [121]. Interestingly, it has been shown that pretreatment with recombinant human erythropoietin (rhEPO) significantly reduced HS and burn-related increases in serum creatinine levels [122,123].

The above mentioned study by Feng et al. [120] not only illustrated that renal failure at 72 h post-burn may relate to ROS-mediated late inhibition of Akt phosphorylation (Fig. 2), but that administration of Akt inhibitors exacerbated renal damage. The role of Akt pathway in AKI was also implicated in animal models utilizing renal clamping and reperfusion injury, but not as extensively as in models of HS. Within 30 min Akt and Bad phosphorylation was increased in kidneys from ischemic mice [124]. Conversely, Akt phosphorylation in the kidney was not increased in HS rats 4 h after reperfusion when compared to sham animals [78]. These differences may relate to observations that phosphorylation events occur rapidly and therefore signal may have been lost by 4 h following trauma, indicating that timing of therapeutic intervention may be relevant. These authors did indicate that HS induces cross talk between Akt and NF-κB signaling pathways by inhibiting the IκB kinase (IKK) complex with 1 mg/mL/kg i.v. infusion of the molecular inhibitor IKK16 (Fig. 2) [78]. Treatment with IKK16 significantly enhanced Akt phosphorylation and activity in the kidney of HS rats. The IKK complex phosphorylates IκBα thereby facilitating the release and translocation of NF-κB to the nucleus. They also demonstrated that IKK16 reduced creatinine and MPO positive cells in the kidney after HS, but this has not been identified after burn injuries.

Caspase expression as mentioned previously is induced with burn and HS and is often quantified to demonstrate activation of cell apoptosis [123,125,126]. In trauma and HS rats the amount of TUNEL positive cells in the kidney paralleled that of relative caspase 3 and 9 expression [127]. More recently, perturbations in mitochondrial function may also be attributed to open mitochondrial permeability transition pore (MPTP) that deregulate the permeability of the inner mitochondrial membrane as seen in HS rats [60]. Opening of MPTP induces cell death through the release of cytochrome *c* and subsequent caspase activation [128]. Taken together, these studies identify mitochondria, apoptosis and MAPK and Akt pathways related pathways as potential novel targets for treating trauma-induced AKI [117].

7. Therapeutic interventions

Fluid resuscitation is one of the basic treatment strategies for both traumatic hemorrhage and burn trauma [129,130]. Although the majority of studies in animal models of HS have focused on the effects of various fluid resuscitation formulations on hemodynamics and survival [131], a few have included renal function. In a study of asymptomatic pneumonia after HS, pigs resuscitated with LR had significantly lower urine output compared to healthy pigs, suggesting some degree of renal

failure [132]. In addition, the effect of hemorrhage and hypertonic fluid resuscitation in dehydrated animals was postulated to compromise renal function. However, no significant effects were observed on glomerular filtration, renal blood flow or filtration fraction compared in dehydrated animals resuscitated with 2 different fluids after HS [133]. A study of hypertonic fluid resuscitation in a 70–85% scald burn model also did not observe a difference in urine output compared to LR treated sheep [134]. Furthermore, despite an increase in renal edema, no difference in urine output was seen over 48 h in a burn and smoke inhalation sheep model [135]. However, it is suggested that fluid resuscitation alone cannot improve AKI once established. Thus, other approaches are necessary.

Animal models have also been used to improve upon other hospital-based treatments. Some of these currently implemented treatments include normobaric hyperoxia treatment to enhance oxygen delivery [62]. HS rats (MAP maintained at 30 ± 5 mm Hg) placed in a 1 atm/100% oxygen chamber had reduced numbers of necrotized tubular cells and lower serum creatinine levels when compared with HS mice placed in a 1 atm/20% oxygen chamber. The efficacy of these types of treatments should be investigated further using animal models.

The molecular mechanisms in response to HS or burn-induced AKI gained with animal models also allow investigators to identify and screen novel treatments for preventing or treating AKI. A quintessential example of this was recently published in Scientific Reports [136]. In this study, melatonin was shown to reverse burn-induced: increases in serum creatinine and BUN; increases in renal MDA, IL-1β, TNF, ICAM-1, Bax, caspase 3, and TUNEL staining; and decreases in renal GSH, SOD, and bcl-2. However, the treatment in this case (i.e., melatonin) is not the novel aspect of the study and, as mentioned earlier, single therapies may not be sufficient for preventing HS- or burn-induced AKI. What this study did identify, however, is the crucial role of sirtuin-1 and its deacetylase activity on p53, p65, and FOXO1. This now identifies other potential pathways that can be leveraged for exerting beneficial effects on apoptosis, inflammation, and oxidative stress.

Due to the multifactorial nature of AKI-induction after trauma (either HS or burn), either a cocktail of pharmacotherapies, or perhaps a biologic therapy that exerts pleiotropic effects may be the most beneficial. For example, stem cell therapy has been touted for some time in a variety of diseases including burn and sepsis [137]. Animal models have examined this possibility in burn-induced AKI as well. One study utilized umbilical-cord derived mesenchymal stem cells (MSCs) in a 20% TBSA scald rat over the course of weeks [138]. Although untreated animals displayed 100% mortality in this study, MSC treatment reduced infiltration of inflammatory cells, normalized serum creatinine levels and improved survival. While baseline creatinine levels were not reported, these authors did show that apoptosis in the kidney was also reduced with MSC treatment. While the mechanisms responsible were not elucidated, it appears that anti-inflammatory properties of MSCs may be involved. Promising therapies such as MSCs should be studied further for their potential use.

Use of antioxidants as a protective measure against traumatic injury has also received much attention. As mentioned previously, the excess production of ROS and RNS generated from HS can be scavenged by various antioxidant defense systems thereby minimizing the impact of the inflammatory cascade. Crocetin, an antioxidant of the carotenoid family reduced iNOS expression and subsequent NO production in HS rats [58]. Crocetin prevented the HS-induced MDA spike and alleviated AKI as demonstrated by reduced creatinine. However authors did not report on Akt signaling mechanisms [58]. Additionally, the antioxidant activity of *Ginkgo biloba* extract reduced serum TNF-α, MPO activity, and creatinine levels in burned rats [71] and in unilaterally nephrectomized rats subjected to renal pedicle occlusion and reperfusion [139]. Others have targeted the mitochondria in an effort to prevent apoptosis. For example, cyclosporine A (CsA), a molecule that inhibits the opening of MPTP has shown benefit after HS. Polytrauma including HS + femur fracture in rats administered 5 mg/kg CsA had 56% survival rate compared with 25% in rats who only received lactated Ringer's (LR) solution [60]. However, much remains to learn regarding the most effective antioxidants and the timing of treatment.

Other researchers have successfully targeted hormones and their functions in an effort to enhance the repair process. One of the most studied is erythropoietin (Fig. 2), which is a HIF-1 induced hormone that maintains survival of erythroid progenitor cells. Rats treated with erythropoietin (EPO) following HS but before resuscitation, had lower creatinine and urea nitrogen serum levels which may have partially contributed to decreased caspase activity [123]. Similarly, erythropoietin injected in rats 5 min prior to burn reduced caspase 3 activity, serum levels of TNF-α, IL-6, and IL-1β and creatinine [122]. It should be noted that EPO administered in this study was prior to injury, which is not applicable to the clinical treatment of traumatic injury. In this regard, it may not be surprising that resuscitation requirements and mortality were not changed in clinical trials of rhEPO administration to burn patients [140], although AKI was not addressed.

8. Conclusion

No review is completely comprehensive, and the review presented here has focused on the development of AKI in relation to hemorrhagic shock and burn trauma. While there may be lessons learned from other models such as reports that include renal artery clamping or chemical-induced AKI, these studies should be interpreted with caution. Moreover, this review has focused on metabolic and immunological aspects of AKI development after traumatic injury that have been derived from HS and burn trauma animal models. While a number of hemorrhage or burn studies discussed focused on other (or multiple) organs, only a few were focused on renal dysfunction. Nevertheless, examining these studies identified some key molecular mechanisms that implicated inflammation and metabolic derangements in the disease progression including oxidant and nitrosative stress markers, cytokines, toll-like receptors, PAMPs, DAMPs, and complement. In addition, there is much interest in the role of mitochondrial damage and apoptosis in development and progression of AKI.

Considering the weaknesses in the current state of the literature discussed above, more research needs to be performed in a methodical fashion. For example, in our review we presented evidence for the role of p38MAPK in AKI, but additional mechanistic studies are needed to distinguish between true AKI and the natural physiologic response of the kidney to HS or burn injury that would not progress to chronic kidney injury. Rodent models, with a specific emphasis on conditional knockout models, as well as cell culture studies could be utilized as a screening tool for identifying both mechanistic pathways and therapeutic targets. Such knockout models could focus on specific pathways in the kidney (and even specific regions within the kidney) to examine which aspects of inflammation, etc. are essential to the development of AKI. An example could be the previously mentioned p38 pathway, which has an extensive downstream signaling network. Also, upstream p38 inhibition has been attempted in rheumatoid arthritis, but was discontinued largely due to liver toxicity. However, this might not exclude trauma from its potential therapeutic repertoire due to the acute nature of burn or HS.

If promising targets have been identified, large animal models could then be used for a second stage analysis of efficacy in different disease states. Large animals such as swine, are not only relatively similarly sized to humans, with similar biochemical values (i.e., creatinine), their response to trauma is also more akin to that seen clinically. Of note we have recently developed a swine burn model where indices of AKI have been observed by 2 h after injury [41]. We are planning to utilize this model in the future to investigate various treatment strategies to include the use of antioxidants, mitochondrial protective agents, complement inhibitors and stem cells, mentioned in this review.

In addition, the limitation of the various approaches to identify AKI is being supplanted by incorporation of multiple aspects of kidney

dysfunction to include KDIGO criteria. The result is progress towards improving translatability of animal studies to the clinical setting to improve treatment strategies for trauma-induced AKI. Certainly further research is necessary to fully understand mechanisms of renal injury induced by trauma. Overall, future strategies that incorporate multiple treatments, including biologics and cell therapies with pleiotropic effects, will undoubtedly be identified for the prevention and treatment of trauma-induced AKI.

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Resuscitation Strategies for Burn Injuries Sustained in Austere Environments to Improve Renal Perfusion and Function

Log Number: MB150175

Award Number: W81XWH-16-2-0041

PI: David Burmeister **Org: The Geneva Foundation/USAISR** **Award Amount:** $749,999

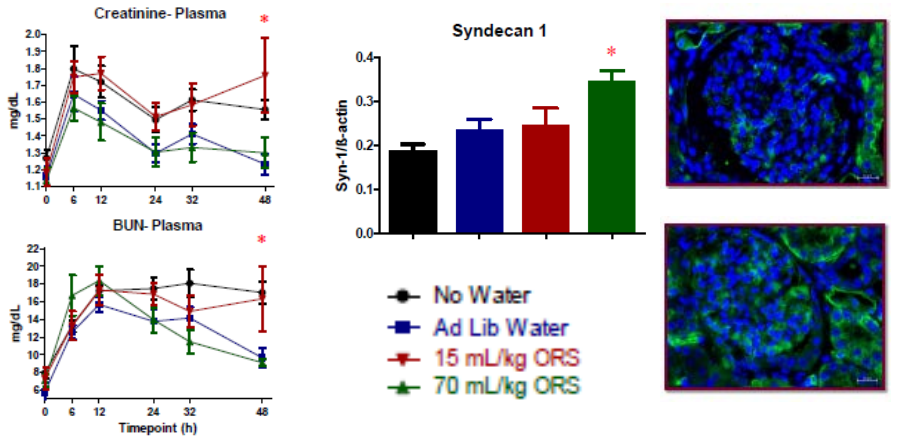
Study Research Aims

Our overall hypothesis is that oral or intravenous resuscitation results in distinct improvements in burn-induced SIRS, MOD and AKI.

- **Compare a standard dose of oral resuscitation solution (ORS) with a lower dose, and fluid deprivation to determine 2 day survival, inflammatory responses and renal function.**
- **Compare a low volume i.v. resuscitation using different fluids with the clinical standard of care for ensuing effects on SIRS, MOD and AKI.**

Approach

To accomplish this we will utilize an established and novel porcine large TBSA burn model that used minimal treatment interventions, with various resuscitation paradigms. A negative control (no fluids) and a positive control (i.v. resuscitation as clinical standard of care) will be compared with resuscitation strategies that are feasible in prolonged field care scenarios. The main outputs are physiological parameters, systemic and local inflammation, and renal perfusion and function.



With sufficient volumes, oral fluids ameliorate burn-induced AKI. Additionally, oral rehydration solutions from the World Health Organization are more protective of the renal glycocalyx , with increased renal Syndecan 1 expression, and more lectin staining in the glomerulus (bottom) compared to water (top right)

Timeline and Cost

Activities	FY 17 Q1	FY 17 Q2	FY 17 Q3	FY 17 Q4	FY 18 Q1	FY 18 Q2	FY 18 Q3	FY 18 Q4
Identify the effect of oral resuscitation on renal perfusion and AKI.								
Identify the effect of oral resuscitation on systemic and local inflammation.								
Identify changes in renal perfusion and function after i.v. resuscitation with the modified Brooke Formula versus a limited resuscitation volume paradigm.								
Identify the effects of a limited volume of 2 different colloids: 5% albumin and fresh frozen plasma (FFP) on SIRS and MOD.								
Proposal Budget 750k	$387.4K				$362.5K			

Updated: OCT 16, 2017

Goals/Milestones

FY17 Goals –

Examine the effect of resuscitation on SIRS/AKI in the above model.

- ✓ Further characterize AKI after water deprivation.
- ✓ Determine the effects of oral resuscitation on SIRS, MOD and AKI.
- ❑ Elucidate the interplay between systemic and renal inflammation.

FY18 Goals – Compare resuscitation strategies.

- ❑ Determine the "rescue" effect of clinical standard of care (LR as per the Modified Brooke Formula).
- ❑ Compare the effects of limited volumes of i.v. resuscitation with LR to Fresh Frozen Plasma, and 5% albumin.
- ❑ Determine the effects of i.v. resuscitation on circulating and local cytokines *ex vivo*.

Comments/Challenges/Issues/Concerns

- None to report

Budget Expenditure as of

Projected Expenditure: $258,267

Actual Expenditure: $ 182,839